## A. List of Publications:

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<th>Sr. No.</th>
<th>Title of the Paper</th>
<th>Name of the Journal</th>
<th>Author and Year of Publication</th>
<th>ISSN/ISBN No.</th>
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<td>3</td>
<td>ICT - A rapid, innovative but simple technique for malaria diagnosis</td>
<td>International Journal of Pharmacy and Life Sciences (IJPLS)</td>
<td>Panchal and Desai (Apr. 2012)</td>
<td>ISSN: 0976-7126</td>
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## B. List of Presentations:

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<th>Author and Year</th>
<th>Name of the Conference</th>
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<td>1</td>
<td>ICT - A rapid, innovative but simple technique for malaria diagnosis</td>
<td>Panchal and Desai (2011)</td>
<td>International Conference on Microbial Biotechnology for Sustainable Development (AMI-2011) Panjab University, Chandigarh</td>
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Short communication

Evaluation of one Rapid Method for Diagnosis of Malaria
The Optional and Better Replacement of Microscopy

Panchal Hetal K. and Desai Pratibha B.

1Dolat Usha Institute of Applied Sciences, Valsad; Veer Nariman South Gujarat University, Surat, Gujarat, INDIA
2Shree Ram Krishna Institute of Comp. Edu. and Applied Sciences; Veer Nariman South Gujarat University, Surat, Gujarat, INDIA

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Abstract

Microscopy has been the most trustable technique for the diagnosis of malaria in India. Reduction of morbidity and mortality rate of malaria highly influenced by earlier and proper diagnosis. This study was carried out at Valsad, Gujarat. It involved use of microscopy i.e. field’s stain and detection of Plasmodium falciparum - HRP II antigen, Plasmodium vivax - pLDH antigen detection by one rapid diagnostic Test (RDT) SD Bioline. Present study was carried out from 966 EDTA anticoagulated samples collected from clinical laboratories and hospitals of Valsad. Microscopic examinations of stained thick and thin films, shows 8.39%, 13.97%, 0.21% were detected as Plasmodium falciparum, P. vivax and mix respectively. Whereas with Rapid Diagnostic test using HRP II, p-LDH antigens 9.05% and 13.87% were detected as P. falciparum, P. vivax respectively. The study shows reasonable harmony between microscopy and RDT. Among two methods RDT was found to have high sensitivity (97.70%) and specificity (98.93%) compared to microscopy. Though the microscopic method is cost effective but laborious and needs an expertise. The RDT results were highly accurate and can be used where microscopy is inaccurate or in case of unavailability of expert.

Keywords: Malaria, malaria diagnosis, rdt, microscopy.

Introduction

Malaria is most important parasitic disease in tropical areas. Around 300 million malaria cases reported each year in the world, causes 1 to 3 million deaths. Nearly 3 billion people lives with the risk of malaria. In India during running year 2011 total malaria cases reported were 336,545. Among them 53.75% were due to Plasmodium falciparum and rest of the cases were due to P. vivax. The disease is caused by Plasmodium species namely P. falciparum, P. vivax, P. ovale and P. malariae transmitted through biting of female Anopheles mosquito. Valsad district of Gujarat state, India is considered as one of the malaria endemic area.

Microscopic examination of thick and thin blood smears stained with Romanovsky’s stain is the most common technique to diagnose malaria since last hundred years. Microscopy continues to be the gold standard for identification of Plasmodium species in the laboratories. The method is easy to apply and cost effective in the laboratories where skilled professionals are available who can even detect very low level of parasite like 10 to 50 parasites/μl. So the sensitivity of microscopy may fluctuate depending upon the skill of technician. In these consequences WHO has recognized the need for simple and cost effective diagnostic test for malaria to overcome the deficiencies of microscopy and clinical diagnosis. To overcome this problem one most easy, cheaper, faster and reliable method available is Rapid Diagnostic Test (RDT). RDT detects P. falciparum Histidine-Rich-Protein II (HRP II) antigen and parasite Lactate Dehydrogenase (pLDH) antigen present in all four Plasmodium species. It does not require any special equipment and give results within 15 to 30 minutes. To estimate the impact of RDT SD Bioline on malaria diagnosis, we analyzed all samples to see the difference between conventional diagnostic microscopy and RDT SD Bioline.

Material and Methods

Total 966 samples were collected from various clinical laboratories of Valsad, Gujarat during December 2010 and July to September 2011. Approximately 1 ml blood sample was collected from each patient in a vacutainer containing an anticoagulant EDTA. All samples were tested by both diagnostic methods microscopy and RDT. Thick and thin smears were prepared on slide, stained with Field’s stain B and A for 5 and 12 seconds respectively. Thick smears were used to confirm malaria and to count parasites/μl. Smears were considered negative if no parasite was observed in 200 consecutive fields of thick smear in oil immersion objective. Parasites were counted against 200 to 500 leucocytes. For the parasite estimation it was assumed that 8000 leucocytes present in 1 μl of blood. Thin smears were used to identify and differentiate parasites, RDT SD Bioline malaria antigen detection test was purchased from SD Bio Standard Diagnostic Pvt. Ltd. The test cassette
contains a membrane strip, precoated with one monoclonal antibody against Plasmodium falciparum HRP II antigen and other polyclonal antibodies specific to pLDH of all 4 human malaria Plasmodium species as two separate lines. The test is one step, rapid, qualitative and differential for Plasmodium falciparum and Plasmodium vivax, the two prominent parasites found in India. For all samples, the cassettes were removed from pouch, approximately 5µl blood samples were placed in small, circular wells with loop, and 4 drops of assay diluent were placed in square assay diluent wells. Results were recorded at the end of 15 minutes and maximum within 30 minutes.

Results and Discussions

Out of 966 blood samples 213 were recognized positive by both tests. Total 218 and 221 cases were found positive by Microscopy and RDT respectively. Detail results are shown in Table 1 and 2. Figures showing observation of ICT and microscopy are shown as figure 1 and 2 respectively. The sensitivity and specificity of the test found was also high about 97.70% and 98.93% respectively. 08 samples were detected as false positive and 05 samples were detected as false negative. Comparison of microscopy and RDT results are also shown in graph 1.

Table - 1
Results of Microscopy and RDT analysis

<table>
<thead>
<tr>
<th>Technique</th>
<th>Positive for P. f</th>
<th>Positive for P. v</th>
<th>Positive for mix</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>81 (8.39%)</td>
<td>135 (13.97%)</td>
<td>02 (0.21%)</td>
<td>748 (77.43%)</td>
<td>966</td>
</tr>
<tr>
<td>RDT</td>
<td>87 (9.05%)</td>
<td>134 (13.87%)</td>
<td>00 (0.00%)</td>
<td>745 (77.12%)</td>
<td>966</td>
</tr>
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</table>

Table - 2
Results of Microscopy and RDT analysis

<table>
<thead>
<tr>
<th>Microscopy/RDT</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>213</td>
<td>05</td>
<td>218</td>
</tr>
<tr>
<td>Negative</td>
<td>08</td>
<td>740</td>
<td>748</td>
</tr>
<tr>
<td>Total</td>
<td>221</td>
<td>745</td>
<td>966</td>
</tr>
</tbody>
</table>

In this study RDT has shown high level of agreement with microscopy. The sensitivity and specificity of the test was also very high. Out of 08 false negative patients 05 were previously treated with chemoprophylaxis against malaria. Even in microscopic analysis of these samples the parasites/µl counts were as low as like 60, 311, 170, 296, 318 etc. Compared with microscopic diagnosis, the SD Bioline RDT was found false positive in 05 patients. This may be due to persistent antigenemia following treatment of malaria in 03 patients with recent history of malaria. Because in some cases antigenemia may remain positive 3-28 days after disappearance of circulating parasites. Other 02 false positive results may be due to rheumatoid factor, hepatitis etc 16,17,18,19. This may be due to a non specific reaction of rheumatoid factor, hepatitis antigens with coated antibodies.

Our study was valuable because the sample size was quite large about to access the acceptability of RDT. Microscopy involves good time and tough microscopic observation. Skilled professional is required to observe the same. Sometime when parasitemia is very low even a keen observation may lead to false negative diagnosis. It is also not easy to differentiate different Plasmodium species without ample experience. In rural areas where skilled malaria detecting experts are not available diagnosis may be delayed and lead to improper diagnosis of malaria and there
by treatment which some time even lead to death. In this situation alternatively we suggest RDT as optional method to diagnose malaria. RDT can be performed within couple of minutes. It is easy to perform that even a new lab technician or a layman can also perform it and interpret the results. Even the RDT was able to differentiate between P. falciparum and P. vivax. It is unable to differentiate P. vivax, P. ovale and P. malariae but in India malaria is mainly caused due to P. vivax and P. falciparum. The sensitivity and specificity of the test found was also high. It suggests RDT as a better option of microscopy in diagnosis of malaria.

Conclusion

In India malaria is mainly found due to P. falciparum and P. vivax. Both can be differentiated well by RDT. Results obtained by RDT are suggesting that it can be used for malaria diagnosis. It makes diagnosis faster, better and reliable. Even can be used at areas where experts are not available or results are needed in emergency.

Acknowledgment

The authors wish to thank to staff of all clinical laboratories of Valsad district, who gave their valuable support. The authors also wish to thank management and staff of Shree RamKrishna Institute of Computer Education and Applied Sciences, Surat; Dolat Usha Institute of Applied Sciences, Valsad.

References


COMPARATIVE EVALUATION OF LIGHT MICROSCOPY AND TWO RDTs IN DIAGNOSIS OF MALARIA

HETAL K. PANCHAL1, PRATIBHA B. DESAI2
1Department of Microbiology, Dolat Usha Institute of Applied Sciences, Valsad, Gujarat, India.
2Shree RamKrishna Institute of Computer Education and Applied Sciences; Athivalines, Surat, Gujarat, India.
1hetal.mistry1982@gmail.com, 2pdesai54@hotmail.com

ABSTRACT

Accurate diagnosis and correct treatment at proper time is a vital factor for reducing morbidity and mortality rate in malaria, which is very high globally. For malaria diagnosis microscopy is still being considered as “Gold Standard”, but use of rapid Diagnostic Tests (RDT) can improve malaria diagnosis as it is simple, rapid and even not that expensive. Our study was aimed to check performance of two RDT SD Bioline and ParaHIT Total in comparison with microscopy. Total 500 samples were collected from various laboratories of Valsad, Gujarat. All samples were tested by microscopy i.e. Field’s stain and by both rapid tests for detection of Plasmodium falciparum - HRP II antigen, Plasmodium vivax - pLDH/ adolase antigen. By microscopy 37, 55, RDT SD Bioline 41, 53 and By RDT ParaHIT Total 40, 45 Samples were found malaria positive as Plasmodium falciparum and Plasmodium vivax respectively from total 500 samples. Sensitivity and specificity were found 97.82%, 99.02% with SD Bioline and 89.13%, 99.26% with ParaHIT Total respectively. Both RDTs were easy to perform and in making interpretation, we found that SD Bioline was comparatively easier to perform and results were also more reliable with it. Proper choice and diagnosis by RDT may change the present scenario of malaria treatment. It may also decrease unnecessary antimalarial treatment of patients or delay in treatment of malaria patients.

Keywords — Malaria, Malaria diagnosis, ParaHIT Total, SD Bioline, Microscopy.

INTRODUCTION

Malaria has been a problem in India for centuries. Currently, 80.5% of the 109 billion population of India lives in malaria risk areas. Of this, 4.2%, 32.5% and 43.8% live in areas of high, moderate, and low risk to malaria respectively [1,2]. At present, official figures for malaria in India, available at NVBDCP [3] indicate 1.5–2 million confirmed cases and about 1,000 deaths annually [3,4]. During running year 2011 total malaria cases reported were 336,545. Among them 53.75% were due to Plasmodium falciparum and rest of the cases were due to P. vivax [5]. The disease is caused by Plasmodium species namely P. falciparum, P. vivax, P. ovale and P. malariae transmitted through biting of female Anopholes mosquito [6,7]. Valsad district of Gujarat state, India is considered as one of the malaria endemic area [8].

Malaria diagnosis is first suspected on defined features and then confirmed by laboratory tests mainly through microscopy; however now a days a new technique known as Rapid Diagnostics Test (RDT) may change the scenario of malaria diagnostics. There are numerous malaria rapid diagnostic tests that are commercially available [2], all of which detect malaria antigen flowing in blood along a membrane containing specific anti-malaria antibodies. The tests fall into a few basic types depending on which antigen is targeted. Most tests which detect P. falciparum are based on the Histidine-rich protein 2 (HRP-2), which is specific to that species. Other tests detect the parasite enzyme lactate dehydrogenase (pLDH), using either monoclonal antibodies which react with pLDH of all species including P. falciparum or antibodies specific for P. falciparum LDH. Other antigens including adolase and other P. vivax specific tests are in early development or use. A distinction between the HRP-2 and LDH based tests is that HRP-2 may persist in the blood stream for days or weeks after treatment, whereas pLDH is only detected if live parasites are present [10]. Early diagnosis and prompt treatment is one of the key strategies for malaria control. Clinical diagnosis is widely used in areas where laboratory facilities are not available; however, it is unreliable due to the non-specific nature of signs and symptoms of malaria [11,12]. Microscopy still remains the gold standard for laboratory diagnosis of malaria, although it is not accessible and affordable in most peripheral health facilities. Recent advent of rapid diagnostic tests (RDT) for malaria may be a significant step forward in case detection, management and reduction of unnecessary treatment. Such RDT could also be useful in malaria
diagnosis during population-based surveys and to provide immediate treatment based on the results. We performed microscopic diagnosis from Field stained blood smears prepared from all 500 samples and they were further also diagnosed by SD Bioline and ParaHIT Total which were able to differentiate both *Plasmodium falciparum* and *P. vivax*.

**MATERIALS AND METHODS**

Samples were collected from various clinical laboratories of Valsad, Gujarat during July to September 2011. Approximately 1 ml blood sample was collected from 500 patients in a vacutainer containing an anticoagulant EDTA. Each sample was tested by microscopy and both RDTs. In microscopy thick and thin smears were prepared on slide. They were stained with Field’s stain B and A for 5 and 12 seconds respectively [13], Thick smears were used to confirm malaria and to count parasites/μl. Smears were considered negative if no parasite was observed in 200 consecutive fields of thick smear in oil immersion objective. Parasites were counted against 200 to 500 leucocytes. For the parasite estimation it was assumed that 8000 leucocytes present in 1 μl of blood [14,15]. Thin smears were used to identify and differentiate parasites. RDT kits ParaHIT Total and SD Bioline were obtained from Span Diagnostics Pvt. Ltd. and SD Biostandard Pvt. Ltd. The kits were stored at room temperature until use. ParaHIT Total was in dipstick format and SD Bioline was a cassette. Both RDT contains a nitrocellulose membrane coated with anti HRP II antibodies, anti pLDH/adolase and anti mouse antisera as 3 parallel non visible lines. For RDT ParaHIT Total, 8 μl of blood sample was blotted on the sample pad just below the arrow on the dipstick. The dipstick with the arrow pointing downwards was dipped in 4 drops of reaction buffer taken in a clean test tubes provided along with the kit. Results were recorded at the end of 15 minutes and maximum within 30 minutes. For SD Bioline, the cassette was removed from pouch, approximately 5μl blood samples was placed in small, circular well with loop, and 4 drops of assay diluent were placed in square assay diluent well. Result was recorded at the end of 15 minutes and maximum within 30 minutes. Results in both RDTs were appearing as rose pink color bands. Both the tests were one step, rapid, qualitative and differential for *Plasmodium falciparum* and *Plasmodium vivax*, the two prominent parasites found in India.

**RESULTS**

500 patients were recruited in this study. Out of 500, 224 (44.8%) were female and 276 (55.2%) were male including 09 children below 8 year age. Table I shows the no. of cases detected as malaria by all 3 methods microscopy, RDT SD Bioline, RDT ParaHIT Total. Table II and Table III shows comparative results of microscopy with RDTs. Sensitivity, specificity, negative predictive value and positive predictive values are given below in Table IV. Fig. 1 and Fig. 2 shows microscopic and RDT results respectively.

| Table I |
| Results of Microscopy and RDTs analysis |

<table>
<thead>
<tr>
<th>Technique</th>
<th>Positive for P. f</th>
<th>Positive for P. v</th>
<th>Total Positive</th>
<th>Negative</th>
<th>Total</th>
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<tbody>
<tr>
<td>Microscopy</td>
<td>37</td>
<td>55</td>
<td>92</td>
<td>408</td>
<td>500</td>
</tr>
<tr>
<td>RDT SD Bioline</td>
<td>41</td>
<td>53</td>
<td>94</td>
<td>406</td>
<td>500</td>
</tr>
<tr>
<td>RDT ParaHIT Total</td>
<td>40</td>
<td>45</td>
<td>85</td>
<td>415</td>
<td>500</td>
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| Table II |
| Comparative results of Microscopy and RDT SD Bioline |

<table>
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<th>RDT/ Microscopy</th>
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<th>Total</th>
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<td>Positive</td>
<td>90</td>
<td>04</td>
<td>94</td>
</tr>
<tr>
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<td>406</td>
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<tr>
<td>Total</td>
<td>92</td>
<td>408</td>
<td>500</td>
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Table III
Comparative results of Microscopy and RDT Para HIT Total

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<tr>
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<tr>
<td>Total</td>
<td>92</td>
<td>408</td>
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Table IV
Results of various RDT parameters

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<th>Parameter/RDT Name</th>
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<th>RDT Para HIT Total</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>97.82%</td>
<td>89.13%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.02%</td>
<td>99.26%</td>
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<td>Negative predictive value</td>
<td>99.50%</td>
<td>97.60%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>95.74%</td>
<td>96.47%</td>
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Fig. 1 Microscopic Observation of Malarial Parasites

Fig. 2 Rapid Diagnostic Test Results

DISCUSSION

Microscopy remains the gold standard for malaria diagnosis, however due to reasons such as lack of skilled laboratory personnel and unavailability of microscopes and reagents, the best diagnostic modality at the moment would be malaria rapid diagnostic tests which can help in the over- and/or mis-diagnosis of malaria and patients' mismanagement who actually have other diseases than malaria. Even sometime delay in diagnosis worsens the situation of malaria patients. Immediate availability of results may change the scenario. To check the applicability we use two different RDT. Both RDT were good at results but SD Bioline was comparatively much better in performing interpretation and results than Para HIT Total. *Plasmodium falciparum* cases were diagnosed well by both RDT even with very low level of parasitemia like 56, 103, 212 etc. Mainly the problem was encountered in diagnosis of *P. vivax* suggesting need of improvement in RDTs in detecting *P. vivax*. Sensitivity and specificity recorded were also high with SD Bioline. Our study suggests that proper choice of RDT can highly improve the malaria diagnostics. False positive results found in some cases by both RDT. This may be due to persistent antigenemia following
treatment of malaria in patients with recent history of malaria. Because in some cases antigenemia may remain positive 3-28 days after disappearance of circulating parasites \cite{16}. Sometime false positive results may be due to a non specific reaction of rheumatoid factor, hepatitis antigens with coated antibodies \cite{17,18,19,20}.

Like our study, reports from elsewhere indicated that RDTs have shown a comparable level of accuracy to microscopy in clinical settings \cite{21,22}. Some other scientists \cite{10} reported very low level of accuracy with RDTs. But that may be due to low parasitemia or mishandling of RDT. RDTs usually have a capacity to detect 100 parasites/µl of blood \cite{21,24}, but in our study RDTs were able to detect very low parasitemia especially in case of \textit{P. falciparum}.

CONCLUSION

Delay in diagnosis and there by treatment may make the situation of malaria patient worst. Use of RDT can give immediate results. Proper choice of RDT may become helpful to avoid unnecessary antimalarial treatment, when patient is not suffering from malaria or may fasten the treatment of actual malaria patients and there by provide a good strategy of reducing morbidity and mortality rate.

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**International Journal of Pharmacy & Life Sciences**

**ICT - A rapid, innovative but simple technique for malaria diagnosis**

Hetal K. Panchal and Pratibha B. Desai

1, Dolat Usha Institute of Applied Sciences, Valsad, Veer Narmad South Gujarat University, Surat, (Gujarat) - India

2, Shree RamKrishna Institute of Computer Education and Applied Sciences, Veer Narmad South Gujarat University, Surat, (Gujarat) – India

**Abstract**

Early, proper and accurate diagnosis plays an important role in case of malaria. Usually malarial diagnosis is done using clinical criteria and/or microscopy. Immunochromatographic tests (ICTs) are now a days widely available and recognized as an alternative method for diagnosis of the malaria. In this study we performed microscopy i.e. Field’s stain and ICT with the help of ParaHIT Total for 897 samples, collected from various clinical laboratories of Valsad district, Gujarat, India. Among them with microscopy 203 but with ICT 198 samples were found malaria positive. In case of microscopy *Plasmodium vivax* (137), *P. falciparum* (65) and in (01) case mix infection was found where as by ICT 65 samples were recognized as *P. falciparum*, and 133 found positive as *P. vivax*. Results of ICT were immediately informed to physicians/patients for faster treatment. Unlike microscopy it is very easy to perform even a layman-nontechnical person can perform it. Even the specificity and sensitivity of dipstick test was found high. Simplicity, easiness and quick results were three important characteristics of ICT which will make it method of choice even in areas where adequate healthcare facilities are not properly available.

Key-Words: Malaria, Malaria diagnosis, ICT, Microscopy.

**Introduction**

Every year 500 million people infected with malaria and 2.5 million people died of it. Malaria has been a major parasitic, communicable tropical disease transmitted by the *Anopheles* mosquito and caused by four Plasmodium species namely *Plasmodium vivax, P. falciparum, P. ovale, P. malariae*. Among them *P. vivax, P. falciparum* are common in India. The re-emergence of malaria has been reported in several countries such as India, Peru, China and Korea. It has become a serious health problem in these countries. Valsad district of Gujarat state, India is considered as one of the malaria endemic area. Even in rural areas proper adequate healthcare facilities are not properly available in India at some places. Early diagnosis and vital treatment are keys to address morbidity and mortality due to malaria. Microscopy remains the gold standard for detection of malaria as it can provide information on both the species of parasite and density of parasite.

Fundamental to improving the care of patients infected with malaria is proper and accurate diagnosis in order to prevent excess morbidity and mortality while avoiding unnecessary use of antimalarial agents and minimizing the spread of resistance to antimalarial drugs. Diagnostic strategies need to be effective not only in resource limited areas where malaria has a substantial burden on society but also in developed countries where expertise in the diagnosis of malaria is frequently lacking.

Immunochromatographic Tests (ICTs) are now a days widely available as a modern technique of malaria rapid diagnostic, which is a faster method, easy to perform, does not require any costlier equipment and give results within 15 to 30 minutes. Malaria antigens currently targeted by ICTs are Histidine-Rich-Protein II (HRP II) for *P. falciparum*, parasite Lactate Dehydrogenase (pLDH) and adolase for other species. It has been estimated that 16 million ICTs were delivered in 2006 all over the world, of which 10.8 million were in Africa and 2.8 million in India. We performed microscopic diagnosis from Field stained blood smears prepared from all 897 samples and they were further also diagnosed by ParaHIT Total,
which can differentiate both *Plasmodium falciparum* and *Plasmodium vivax*.

**Material and methods**

The fresh flowers of *Rakta Karpasa* (*Gossypium arboretum*) are used to confirm malaria and to count parasites/µl.

Various blood samples were collected from 897 patients showing symptoms of malaria in between July 2010 to November 2010 at various clinical laboratories and hospitals of Valsad district, Gujarat, India. All samples were immediately tested with ICT ParaHIT *Total*. The kits were obtained from Span Diagnostics, Surat, India. The kits were stored at room temperature until use.

Thick and thin smears were prepared on slide at the same time. They were stained with Field’s stain B and A for 5 and 12 seconds respectively. Thick smears were used to confirm malaria and to count parasites/µl. Smears were considered negative, if no parasites were observed in 200 consecutive fields of thick smear in oil immersion objective. Parasites were counted against 200 to 500 leucocytes. For the parasite estimation it was assumed that 8000 leucocytes present in 1 µl of blood.

Thin smears were used to identify and differentiate parasites.

**Results and Discussion**

203 of 897 patients had malaria infection according to Microscopic diagnosis of blood film examination. 137 had *P. vivax*, 65 had *P. falciparum* and 01 had mix infection with *P. vivax* and *P. falciparum*. 694 cases were diagnosed negative (Table I, Table II). Figures showing observation of ICT and microscopy are shown below as Fig. 1 and Fig. 2 respectively.

Out of 897 samples 198 were diagnosed positive, 65 as *P. falciparum* and 133 as *P. vivax*, where as 699 samples were diagnosed negative by ParaHIT Total RDT (Table I, Table II). The sensitivity and specificity of the test found was also high. Compared with microscopic diagnosis, the ParaHIT Total ICT was found false positive/negative in some patients. The sensitivity and specificity of the test found was also high.

Sometime when parasitemia is very low even a keen observation may lead to false negative diagnosis. It is also not easy to differentiate different *Plasmodium* species without ample experience. In internal rural areas where skilled malaria detecting professionals are not available diagnosis may take longer time and lead to improper diagnosis of malaria and there by treatment which some time even lead to death. In this situation ICT alternatively we suggest ICT as optional method to diagnose malaria.

We were able to give results soon within 5 minutes but as per manufacturers instruction we gave results to patients after 15 minutes so that their treatment can appropriately get start. Even the ICT was able to differentiate between *P. falciparum* and *P. vivax* and even a layman can perform and interpret the results.

Microscopy at least needs nearly an hour and laborious procedure and involve tough microscopic observation. Skilled professional is required to observe the same.

There is a difficulty in performing microscopy so, alternatively we suggest ICT as optional method to replace of microscopy in diagnosis of malaria.

Microscopy is a life threatening infection influences most developed as well as under developed countries of the world with regions of the world lacking basic health infrastructure. High burden of disease, emerging antimalarial drug resistance and broad implementation of ACT are placing greater emphasis on rapid and accurate diagnosis of patients infected with malaria.

There is a difficulty in performing microscopy so, alternative diagnosis method is required. The possible answer we found is ICT which no more require any skilled personnel like microscopy and is a faster cheaper and reliable as we found it. Even any lay man can perform it after giving him a simple understanding.

A highly effective ICT could avert over 100,000 malaria related deaths and about 400 million unnecessary treatments. ICT do not eliminate the need of microscopy until a new gold standard is developed, but Malaria ICTs are ushering in a new era of diagnosis to improve the overall global health care system.
Acknowledgements

The authors wish to thank to staff of all clinical laboratories of Valsad district, who gave their valuable support. The authors also wish to thank management and staff of Shree Ram Krishna Institute of Computer Education and Applied Sciences, Surat; Dolat Usha Institute of Applied Sciences, Valsad.

References

Table 1: Results of microscopic and ICT analysis

<table>
<thead>
<tr>
<th>Technique</th>
<th>Positive for P. f</th>
<th>Positive for P. v</th>
<th>Positive for mix</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>65</td>
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<td>699</td>
<td>897</td>
</tr>
</tbody>
</table>

Table 2: 2×2 Results of microscopic and ICT analysis

<table>
<thead>
<tr>
<th>ICT/Microscopy</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>187</td>
<td>11</td>
<td>198</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>683</td>
<td>699</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>694</td>
<td>897</td>
</tr>
</tbody>
</table>

Fig. 1: Rapid Diagnostic Test Results

Fig. 2: Microscopic Observation of Malarial Parasites